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RATE ACCOMPANYING THE CONDI-
TIONED STATE INDUCED BY
MORPHINE**

A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

By
DOROTHY PALMER GULLIKSEN

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CHANGES IN THE BASAL METABOLIC RATE ACCOMPANYING THE CONDITIONED STATE INDUCED BY MORPHINE

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Subcutaneous injection of morphine into dogs produces in a few minutes salivation, retching and vomiting. After seven or eight daily administrations, Collins and Tatum (1925) found that the animals would salivate and sometimes vomit before the drug was given. The conditions immediately preceding the injection such as the sight of the experimenter or of the hypodermic needle called forth the reaction. The salivary secretion occurring during this reflex was quantitatively studied by Kleitman and Crisler (1927), who associated the injection of morphine with a certain definite set of conditions which could be maintained for an hour or more before the injection. It was found that after a few daily injections of morphine, salivation occurred before the injection in increasing amounts from day to day until a certain level was reached. Accompanying this conditioned salivation, it was also observed that panting, whining and occasionally vomiting occurred. The symptom complex of which these objective signs are a part was called nausea by the writers.

This state of nausea resembles very markedly some of the responses classed as emotional or excitement reactions by Cannon and Britton (1926). Cats when restrained in a holder showed uneasy twitching of the tail, occasional mewling and turning of the head, minor signs of excitement, the authors state. The studies presented in this paper are an attempt to determine whether the conditioned state, induced by morphine, is accompanied by an increase in metabolism over the basal metabolic rate.

It is asserted that minor signs of excitement in the cat as well as strong emotion are accompanied by increased adrenal secretion (Cannon and Britton, 1926). It has also been shown that intravenous injection of adrenalin in doses claimed to be within a physiological range, lead to increases over the basal metabolism (Boothby and Sandiford, 1923). Therefore, if increases over the basal metabolism occurred during the conditioned morphine excitement, it seemed important to determine whether increased adrenal secretion was an important factor in producing the augmented metabolism.

METHOD. 1. *Apparatus.* The Benedict portable respiratory apparatus

was used with the mask for covering the dog's muzzle as described by Kunde (1923) with the addition of a small outlet on its under surface connected by rubber tubing with an S-shaped glass tube containing water in the bend of the tube. Saliva could flow off by way of this tube while air could not enter the system. The joint between nose and mask was tightened by fastening a rubber band (about two inches wide) over the line of contact (Kunde et al., 1930). A graphic record of the oxygen consumption and respiration was made by a writing point fastened to the spirometer bell recording on a smoked drum. The apparatus was tested for leaks up to the point of junction of the mask with the dog by placing a 5 gram weight on the spirometer bell, placing a small glass bottle in the opening of the rubber dam, and opening the valve so that the mixture of oxygen and air in the system circulated through the mask being forced through the circuit by the rotary blower. If the writing point on the bell did not move during five minutes, the machine was judged to have no leak. Duplicate basal metabolism tests with a small weight on the spirometer bell were also made to test for leaks.

2. *Food and care of dogs.* Five dogs were used, each weighing about 10 kilograms. They were dewormed and kept dry and free from mange. Each dog was fed at least 20 hours before a test with a standard diet of 200 grams of meat, 250 cc. milk, and from 100 to 200 grams white bread, the amount of bread being determined by what the animal would eat regularly day after day without leaving any. This amount would usually be less in warm weather than in cold weather. Two to three tablespoons of bone meal were put into the mixture. Each dog was allowed to run about alone in an open pen or room each day and defecation permitted before being brought to the test room.

3. *Procedure.* Daily determinations of metabolism were made until the average metabolism for a two-week period lay within the range found by Kunde and Steinhaus (1926) in their study of basal metabolism in normal dogs. For Doctor Kunde's dogs, the average basal metabolisms ranged from 686.0 to 843.0 cal. per square meter per 24 hours, the average value being 777.5 cal. per square meter per 24 hours. It would often require weeks of training before this basal level was reached. Each day the dog was weighed, brought to the metabolism room, and rested for forty-five minutes on a table. Then a fifteen minute test was made with the room quiet. At the close of the test pulse rate and rectal temperature were recorded. The animal was then taken to his cage and fed, the next test being made at least 20 hours after feeding.

Following the two week control period, the conditioned reflex was established in the following manner. A metabolism determination was made every other day, and immediately at the close of each test, while the dog's muzzle was still in the mask, an injection of $\frac{1}{2}$ grain of morphine sulphate

was given subcutaneously. The dog was held quietly on the table. Three or four minutes later the dog would usually attempt to draw his head from the mask, and would retch, or vomit mucus. The morphine stupor then followed. The dog's head was then removed from the mask, and he was taken to his cage. The dog was given food immediately, but usually would not eat for several hours. To prevent the direct effect of food or of morphine on the next metabolism test, the dog was not given a metabolism test next day. He was fed early the day after an injection and 20 hours later was taken to the metabolism room for a metabolism test immediately followed by injection of morphine.

After about three of these tests followed by morphine, the dogs would often struggle against having the mask put on, and would salivate somewhat during the test. After a few tests the animal would remain fairly quiet on the table if the experimenter held the dog's legs and head gently in place, a procedure which had no effect when tried during the control determinations of basal metabolism. These determinations of metabolism followed by morphine injections were made every other day for a month. After an interval in which the metabolism returned to the basal level, another control period was taken, followed by another period of morphine tests. It was thought that possibly an excess oxygen consumption during the morphine test period might be due to swallowing of saliva containing air. For this reason, the salivary glands (sub-maxillary, sublingual, parotid and orbital) were removed from two dogs already tested and a control period and a morphine period repeated on them.

Denervation of the adrenal glands was performed next on two new dogs and one dog which had been given three control basal metabolism periods and three morphine test periods. The method of denervation of the adrenals is similar to that outlined by Cannon (1919). The right adrenal gland was removed and also the left lumbar sympathetic chain between the diaphragm and the kidney. Next the thoracic sympathetic chain on the left side was removed for about two inches just above the diaphragm, an incision being made for this purpose between the 10th and 11th ribs.

The animal was then allowed to recover, but was brought to the testing room each day and put through the routine of rest and metabolism testing. After two weeks of control basal metabolism tests, the injections of morphine were made every other day as in the previous experiments. Two other dogs were subjected to the denervation operation and were killed after five weeks for histological examination of the region where the sympathetic chain had been removed to see if regeneration had occurred.

RESULTS. A. In the five dogs studied, the average of the first morphine test period showed the following percentage increase over the average of the preceding basal metabolism control period; unoperated dogs, A, 23.7 per cent; B, 28.0 per cent; C, 13.0 per cent: adrenal operated dogs, D, 22.2

TABLE 1

Summary of control basal metabolism tests and metabolism tests during morphine conditioning periods

DOG	PREVIOUS TREATMENT	SERIES	PERIOD	NUMBER OF TESTS	AVERAGE WEIGHT	AVERAGE CALORIES PER 24 HOURS	AVERAGE CALORIES PER SQUARE METER PER 24 HOURS	TEST AVERAGE MINUS CONTROL AVERAGE PER 24 HOURS	PER CENT INCREASE OF TEST PERIOD AVERAGE OVER CONTROL PERIOD AVERAGE	P
					kgm.			calories		
A	Normal Normal Normal Normal Salivary glands removed Right adrenal gland removed and left adrenal gland dener- vated	I	Control	14	8.6	368.79	782			
		I	Test	14		456.11		87.32	23.7	0.01—
		II	Control	14	9.4	366.32	730			
		II	Test	10		428.69		62.37		0.01—
		III	Control	17	8.9	372.35	775			
		III	Test	9		440.43		68.08		0.01—
		IV	Control	15	8.5	370.38	795			
		IV	Test	13		418.15		47.77		0.01—
B	Normal Normal Normal Normal Salivary glands removed	I	Control	14	11.6	434.42	750			
		I	Test	10		556.03		121.61	28.0	0.01—
		II	Control	14	12.7	447.46	730			
		II	Test	17		444.75		-2.71		0.7+
		III	Control	15	11.8	447.03	750			
		III	Test	10		438.64		-8.39		0.2—
C	Normal Normal	I	Control	25	10.3	434.32	820			
		I	Test	10		490.71		56.39	13.0	0.01—
D	Right adrenal gland removed and left adrenal gland dener- vated	I	Control	13	12.3	494.34	825			
		I	Test	13		604.12		109.78	22.2	0.01—
E	Right adrenal gland removed and left adrenal gland dener- vated	I	Control	15	8.6	333.8	710			
		I	Test	10		446.1		112.3	33.6	0.01—

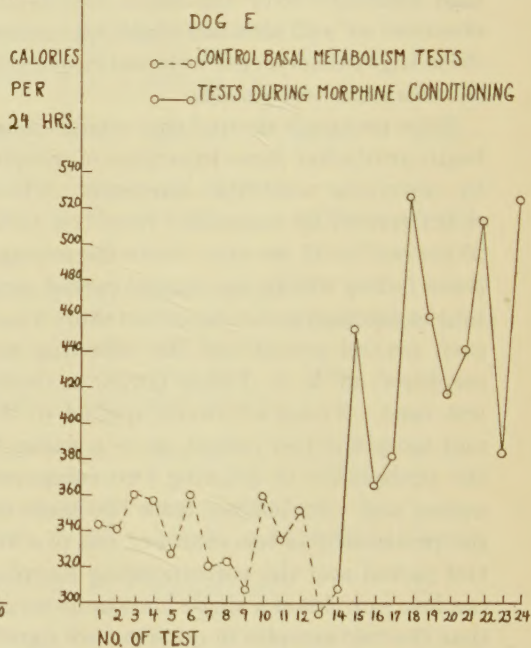
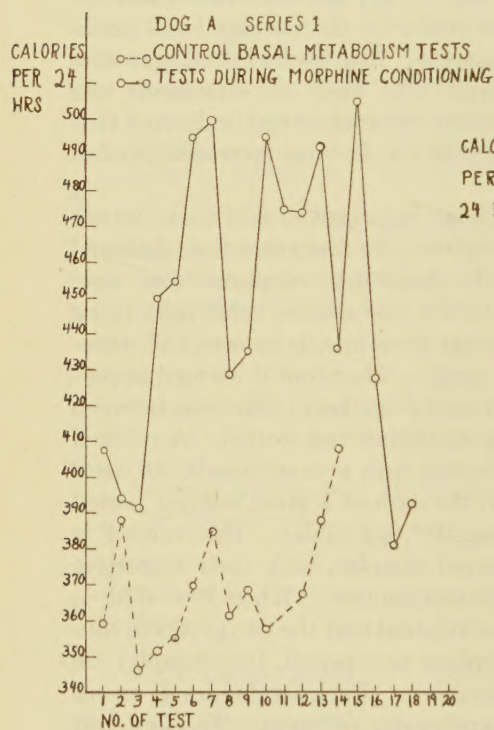
per cent; E, 33.64 per cent. (See table 1.) These figures do not include tests in which the dogs started to rise or made similar marked movements, since it has been shown (Benedict, 1924) that muscular exertion may increase the metabolic rate over the basal level. Tests were retained, however, in which slight movements were visible, since Benedict reports that moving the hand to the face once or twice a minute does not modify the basal metabolism, and that raising the hand to the face every four seconds gives an increase of plus 6 per cent to plus 11 per cent over the average basal metabolic rate. The slight movements were not observed in the dogs as often as every four seconds, and if they had occurred, would not explain the large increases (up to 35 per cent over the average basal metabolic rate) often found in the morphine tests. Also the fact that there were high increases over the basal metabolic rate when no movement was observed as well as when slight movements were observed, indicated that these slight movements were not responsible for the large increases found in the morphine test periods.

Since protocols showed that conditioning, as judged by salivation, did not begin until after three injections of morphine, the first three tests followed by injections were also discarded. The remaining morphine tests were characterized by variability from test to test (see graphs) sometimes being 25 per cent to 35 per cent above the average basal metabolic rate, and sometimes falling within the control period range. Therefore it seemed important to determine whether or not there was any significant difference between each control period and the following morphine test period. A method employed by R. A. Fisher (1928) in dealing with a small number of cases was used. Fisher's formula applied to the data of a given control period and morphine test period, gives a value "P" (see table). This value P is the probability of drawing two compared samples, with their respective means and variabilities, from the same distribution. When $P = 0.01$ —, the probability is less than one out of a hundred that the two groups (a control period and the corresponding morphine test period, for example) are random samples of a single homogeneous group. Such a value for P means that the two samples in question are significantly different. The first test period was in all cases significantly different from the control period as shown by $P = 0.01$ — in each case.

B. *Spontaneous changes in the metabolism accompanying the conditioned response to morphine.* In series 2 where the morphine injections were repeated in dogs A and B, there is evidence for a spontaneous decrease in the metabolism response on repeated conditioning of the animal. In dog B, in both series 2 and 3, the average of the test period is actually lower than the average for the corresponding control period. The values for P indicate that there is little or no difference between the test periods and control periods. In dog A there is less striking a decrease in the response, the

difference between test and control period averages being 87.32 cal. in series 1, and 62.37 cal. and 68.08 cal. respectively in series 2 and 3.

C. *Artefacts due to swallowing air.* The data show that oxygen consumption values were not enlarged by swallowing air with saliva. In dog A, the differences between control period and test periods are practically the same in series 2 (just before removal of the salivary glands) and in series 3 (after removal of the salivary glands). In dog B the average of the test period in series 2 is lower than that of the control period of the same series, even though the salivary glands were present and some salivation was



noted. In this dog, after removal of the salivary glands, in series 3, there is a further slight reduction of the test period average below the control period average, but this would seem insignificant as compared to the spontaneous drop occurring between series 1 and 2 in this same dog.

D. *Adrenal gland denervation.* In view of the spontaneous decrease in the increments over the basal metabolic rate occurring with repeated conditioning of the animals, denervation of the adrenal glands was carried out in two dogs which had never received morphine injections. A comparison was then made of their first metabolism response on morphine conditioning

and the first metabolism responses of the three dogs with adrenal innervation intact. The differences between control period and test period averages in series 1, for the five dogs are: normal dogs, 23.7 per cent, 28.0 per cent and 13 per cent; operated dogs, 22.2 per cent and 33.64 per cent. One of the operated dogs, it is seen, is near the highest normal dogs, and the other operated dog shows the greatest percentage increase of the five dogs studied. It might be expected that if adrenal denervation ruled out a hormone of importance in producing the increased metabolisms of the test periods, dogs D and E (operated) should show considerably less rise over the basal metabolic rate than dog B (unoperated).

A comparison of the morphine test periods in dog A before and after his adrenal glands were denervated, is also relevant. In series 3, before denervation of the adrenals, there is a difference between control and test period of 68.08 cal., while in series 4, after denervation, the difference is 47.77 cal. In view of the spontaneous fall in the response between one series and the next already observed in this dog, and in dog B, the most significant values seem to be those given above, where normal and operated dogs are compared on first being conditioned to morphine.

Two dogs with adrenals denervated were killed five weeks after the last stage of the operation, removal of 1 to 2 inches of thoracic sympathetic chain above the diaphragm. Gross examination showed connective tissue where the chain had been. Histological examination showed no evidence of nerve fibers regenerating in the area. The denervated experimental dogs were showing a definite increase in the metabolism over the basal level an equal time after operation.

DISCUSSION. The results presented indicate that test conditions associated with the giving of morphine induce a state of excitement which is accompanied by marked increases in metabolism over the basal metabolic rate. There are certain practical bearings of this experiment, for it is possible that in taking ordinary basal metabolism determinations on dogs, incidental stimuli occurring during or after the test may lead to disturbances of the basal metabolic rate, and account for some of the variability reported in basal metabolism studies.

Relation of the adrenals to basal metabolic rate. It has been reported by various workers that basal metabolism is lowered by procedures said to remove adrenal secretion. Aub, Forman and Bright (1922a) studied one cat with the right adrenal removed and the left splanchnic nerves cut. They report a fall of 12 per cent in the metabolism below the basal level 37 days after the operation. They conclude that removal of one adrenal and denervation of the remaining adrenal is followed by a slow fall of the metabolic rate. The same workers (1922b) removed both adrenal glands and determined metabolism under urethane anesthesia, and found a "prompt and progressive fall of metabolism which averages 12 per cent."

Since "adrenalin injected intravenously at a physiological rate causes a distinct rise in the metabolism" the conclusion is suggested that the fall in metabolism after adrenalectomy was due to lack of secretion from the adrenal medulla. McIver and Bright (1924) found an average drop of 7 per cent in the metabolism of animals under urethane anesthesia following removal of the adrenals.

Three dogs studied in this paper had the remaining adrenal medulla presumably removed from nervous influences. Two of the three dogs had average basal metabolism values in calories per square meter of surface area, which were in the higher part of the normal range. The third dog had a basal metabolism lower than the other dogs tested, yet well within the normal range. These results do not indicate a lowering of the basal metabolism following the denervation technique employed.

Extra adrenalin and metabolism. It has been reported by Boothby and Sandiford (1923) that adrenalin injected intravenously into dogs increases the metabolism. Adrenalin chloride injected into normal dogs at rates claimed by Cannon and Rapport (1921) to be within physiological limits gave increases of 25 per cent to 35 per cent over the average basal metabolic rate. Hunt and Bright (1926) gave intravenous injections of adrenalin at a rate slightly above the maximum physiological increase as determined by Cannon and Rapport. The peaks of the rises of metabolism averaged 19 per cent over the control level. In a paper by Cannon, Querido, Britton and Bright (1926), it is stated, that "since injected adrenalin increases metabolism, and adrenalectomy decreases it, the inferences seem justified that absence of the medulla and its secretion, rather than absence of the cortex, is responsible for slower metabolic rate after removal of the adrenals; and conversely that secreted adrenin helps to maintain the normal metabolic rate and by a larger output may increase that rate."

According to Cannon and Britton (1926) some of the circumstances which lead to the secretion of adrenalin, as indicated by a faster rate of the denervated heart, are slight muscular contractions, strenuous exertion, minor excitement and strong "emotion," the two latter produced in a cat by the presence of a barking dog. Since in these experiments the liver and thyroid were also denervated, the question arises as to whether or not adrenal secretion has an important function in normal animals.

Some evidence on this point has been presented in a study of animals working on a treadmill (Campos, Cannon, Lundin and Walker, 1929). It was found that if one adrenal was removed and the other denervated, the capacity for work was not decreased as compared with normal dogs. The comment is made that "it was not at all expected that after removal of one adrenal and demedullation of the remaining gland the energy output would be as great as under standard conditions." The authors give the explanation that possibly "the normal animal does not use that (adrenal)

apparatus to any noteworthy degree in routine running, but only in unusual emergencies." However since the dogs were exercised until they refused to run and often were unable to stand, they would seem to be in a situation which would be generally classed as an emergency.

The results reported above also bear on the question of adrenal function in normal animals. Those dogs with one adrenal removed and the other denervated, showed as great an increase over the basal metabolic rate during the conditioned state as did the unoperated dogs. According to the reports of Cannon and Britton, cited above, adrenalin might be expected to be secreted during the conditioned response induced by morphine, but if such a secretion occurred, it was not necessary for the increases over the basal metabolism which were found. It must be assumed that if in the normal dogs adrenalin was secreted in excess of basal amounts during the conditioned excitement period, and caused some of the increased metabolism observed, then in the operated dogs if no excess adrenalin was secreted, its stimulating action on metabolism was completely taken over by some other agency.

CONCLUSIONS

1. Increases in metabolism over the basal metabolic rate accompany the conditioned state induced in dogs by morphine. It is shown that these increases are greater than chance variations from the basal metabolic rate.

2. The degree of increase over the basal metabolic rate during the conditioning period varies from dog to dog.

3. After allowing time for disappearance of the conditioned state a second or third induced conditioned state is accompanied by less increase over the basal metabolic rate.

4. After removal in dogs of one adrenal gland and denervation of the other no decrease in basal metabolic rate occurred during the control periods. During the conditioned state increases over the basal metabolic rate occurred as in normal dogs. Discharge of adrenalin seems not to be a necessary factor in this increase.

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BIBLIOGRAPHY

- AUB, J. C., J. FORMAN AND E. M. BRIGHT. 1922a. *This Journal*, lxi, 326.
1922b. *This Journal*, lxi, 349.
BENEDICT, F. G. AND C. G. BENEDICT. 1924. *Bull. de la Societe Scientifique D'Hygiene Alimentaire*, xii, 1.
BOOTHBY, W. M. AND I. SANDIFORD. 1923. *This Journal*, lxvi, 93.
CAMPOS, F. A. DE M., W. B. CANNON, H. LUNDIN AND T. T. WALKER. 1929. *This Journal*, lxxxvii, 680.
CANNON, W. B. 1919. *This Journal*, l, 399.

- CANNON, W. B. AND S. W. BRITTON. 1926. This Journal, lxxix, 433.
- CANNON, W. B., A. QUERIDO, S. W. BRITTON AND E. M. BRIGHT. 1926. This Journal, lxxix, 466.
- CANNON, W. B. AND D. RAPPORT. 1921. This Journal, lviii, 308.
- COLLINS, K. H. AND A. L. TATUM. 1925. This Journal, lxxiv, 14.
- FISHER, R. A. 1928. Statistical methods for research workers. 2nd ed., London.
- HUNT, H. B. AND E. M. BRIGHT. 1926. This Journal, lxvii, 353.
- KLEITMAN, N. AND G. CRISLER. 1927. This Journal, lxxix, 571.
- KUNDE, M. M. 1923. Journ. Met. Res., iii, 399.
- KUNDE, M. M. AND A. STEINHAUS. 1926. This Journal, lxxviii, 127.
- KUNDE, M. M., ET AL. 1930. This Journal, xcv, 630.
- McIVER, M. A. AND E. M. BRIGHT. 1924. This Journal, lxviii, 622.

